

dedifferentiation. Applicants traverse this rejection and withdrawal thereof⁹⁾ is respectfully requested.

The Examiner's sole basis for this rejection is the incorrect presumption that treatment with dedifferentiation medium for 1-6 days will not result in dedifferentiation. However, as discussed previously and as further discussed below, the Examiner's primary presumption is incorrect. With the presently claimed method, one day culture on dedifferentiation medium is sufficient for dedifferentiation if an explant is cultured with an *Agrobacterium* containing a super binary vector having the virulence region of Ti plasmid A281, left and right border sequences of T-DNA of a Ti plasmid or an Ri plasmid of an *Agrobacterium*, with a desired gene being located between the left and right border sequences. As such, the present invention is not the equivalent of the subject matter of claims 1-14 of the '238 patent and withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. 112, first paragraph

Claims 1-16 have been rejected under 35 U.S.C. §112, first paragraph for lack of enablement. The Examiner asserts that Potrykus sets forth the following five criteria as proof of stable plant/cell transformation.

- 1) serious controls for treatments and analysis;
- 2) a tight correlation between treatment and predicted result;
- 3) tight correlation between physical (e.g. Southern blot) and phenotypic (e.g. enzyme assay data);

4) complete Southern analysis containing the predicted signals in high molecular weight DNA, in hybrid fragments between host DNA and foreign gene, and the complete gene; evidence of the absence of contaminating fragments of DNA or identification of such fragments; and

5) data allowing the discrimination between false positives and correct transformations, as well as genetic and molecular analysis of offspring.

The Examiner asserts that the present specification fails to meet these five criteria for the following reasons. The Examiner asserts that the inability to recover "transgenic tissue" is indicative of a high level of skill in the art. The Examiner further notes that page 31 indicates the inability to produce resistant cells from the scutellum samples and that a 6 kb fragment is too small to be indicative of chromosomal integration.

The Southern analysis data is asserted as being insufficient because it is presented in summary form without actual data being presented along with the control data. Finally, the Examiner asserts that the teachings of Schlappi et al. that undifferentiated meristematic tissues of very young, immature maize embryos were not competent for agroinfection and the disclosure of Hiei et al. are in conflict with the present claims.

Applicants traverse this rejection and withdrawal thereof is respectfully requested.

The present invention is drawn to a method of transforming a monocotyledon comprising contacting an explant that has been cultured with dedifferentiation medium for 1-6 days with an *Agrobacterium* containing a super binary vector having the

virulence region of Ti plasmid A281, left and right border sequences of T-DNA of a Ti plasmid or an Ri plasmid of an *Agrobacterium*, with a desired gene being located between the left and right border sequences.

The Examiner appears to misunderstand both the invention and the specification. The Examiner's misunderstanding is readily apparent from his reliance on various parts of the specification as being indicative of the non-enablement of the invention. For example, the Examiner relies on the following statement on page 30, line 20 of the specification as being indicative of the "inability to recover transgenic tissue."

Although the shoot apex exhibited high rate of introduction of GUS gene after the culture with the *Agrobacterium* strain, after the selection by hygromycin, all tissue died and no tissue resistant to hygromycin were obtained.

However, this sentence has nothing to do with the ability to obtain transgenic tissue with the present invention. The sentence in question describes the results of the Comparative Example using shoot apex tissue, which is outside the scope of the present invention, because shoot apex tissue is not a dedifferentiated or dedifferentiating type of tissue.

The Examiner further questions the chromosomal integration of the DNA. However, as stated on page 44, lines 22-26 and shown in Tables 8 and 9 of the specification, the GUS gene was inherited by progeny according to Mendel's law. This is clear evidence that the GUS gene was integrated into the chromosomal DNA of the host and that the gene was then inherited by the progeny (criteria 5 of Potrykus).

With regard to the criticism of the Southern blot analysis, attached hereto is a copy of the Declaration of co-inventor Dr. HIEI, dated November 9, 1998 and previously submitted in the parent application to address the same point.

Items 2 and 3 of the Declaration address the Southern blot analysis of the transformants. As detailed in Item 2, transformants, controls of non-transformant plants and pTOK233, and progeny of the transformants were all analyzed by Southern hybridization on either restriction digested DNA using the HPT gene and GUS gene as probes or non-digested DNA using the HPT gene as a probe.

The results of the Southern analysis are discussed in Item 3. Figure 3 shows the results of the hybridizing the total DNA with the HPT gene. The HPT hybridization with the control plasmid identifies the gene in a 49.4 kb band. If the gene expression is the result of the Agrobacterium remaining in the rice plants, the transformants should have the same 49.9 kb hybridization band. However, as clearly shown in Figure 3 of the Declaration, with all of the transformants, the HPT probe hybridized with high molecular weight nuclear genomic DNA of the rice plants. As such, it is clear that the T-DNA is inserted into the rice chromosomal DNA.

The results of the Southern analysis of the digested DNA of the primary transformants show one to several copies of the introduced gene being present. See Figure 4. However, when the plasmid pTOK233 control was similarly digested with HindIII and subjected to Southern hybridization, there was only a single constant band of 5.1 kb. See the arrows of Figs. 4 and 6. Clearly, if the Agrobacterium had remained in the rice plants and

somehow been transferred to the progeny, only the single 5.1 kb band seen with the control would be seen with the progeny. However the results shown in Figs. 4 and 6 show bands of varying length with the progeny, when probed with HPT.

The results of the Declaration also detail that the GUS expression and hygromycin resistance segregated in the progeny plants and were inherited in accordance with Mendel's laws of genetic inheritance. This clearly indicates that the genes were not the random association of Agrobacterium, but rather that the genes had been stably inserted into the rice genomic DNA.

These data demonstrate that the Examiner's requirement that the transgenes be associated with high molecular weight DNA has been met and further that the plants have been transformed with insertion of the DNA into the plant genome.

Finally, neither Schlappi et al. nor Hiei et al. are in conflict with the present invention. The present invention requires the use of a super binary vector. The present inventors have found that by using the very efficient super binary vector, culture on dedifferentiation-inducing medium need only take place for 1-6 days. Schlappi et al. does not discuss a super binary vector, therefore there is nothing in conflict in Schlappi et al.

There is further no conflicting disclosure in Hiei et al. Hiei et al. disclose the possible use of a super binary vector but a) Hiei et al. is not limited to the use of super binary vector and therefore must be able to also work with binary vectors that are not super binary vectors and b) Hiei et al. does not state that "with a super binary vector the culture duration must be not less than 7 days" or contain any statements to that affect. If a

binary vector is used, which is not a super binary vector, the culturing on the dedifferentiation medium must be for not less than 7 days. Because Hiei et al. encompass the use of binary vectors which are not super binary vectors the culturing on the dedifferentiation medium must be for not less than 7 days. However, such a requirement does not preclude a subset of binary vectors, i.e. super binary vectors, from being capable of transformation under different conditions.

The Examiner asserts that the teachings of Hiei et al. are in conflict with the present invention. However, a "contradictory" or "conflicting" teaching would be a teaching that with super binary vectors specifically the culturing on the dedifferentiation medium must be for not less than 7 days. There is no such teaching in Hiei et al. The emphasized statement of Hiei et al. that is relied on by the Examiner, that "a super binary vector may preferably be employed in the present invention" is not a conflicting statement with the present claims. Even though a super binary vector may be preferred if the dedifferentiation period is not less than 7 days, there is nothing that precludes the use of a super binary vector specifically with culture conditions on dedifferentiation medium of 1-6 days. As such, the present invention is not in conflict with either Schlappi et al. or Hiei et al.

For the reasons discussed above, the present invention is has been demonstrated as being fully enabled as claimed. Withdrawal of the rejection and allowance of the claims are therefore respectfully requested.

As the above-presented amendments and remarks address and overcome the rejections of the Examiner, withdrawal of the rejections and reconsideration and allowance of the claims are respectfully requested. Should the Examiner have any questions regarding the present application, he is requested to contact MaryAnne Armstrong, PhD (Reg. No. 40,069) in the Washington DC area, at (703) 205-8000.

Pursuant to 37 C.F.R. § 1.17 and 1.136(a), the Applicants respectfully petitioned for a three (3) month extension of time for filing a response in connection with the present application and the required fee of \$890.00 is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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Enclosures: Declaration of Yukoh HIEI